Commentary

Activating calcium release through inositol 1,4,5-trisphosphate receptors without inositol 1,4,5-trisphosphate

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Cytosolic calcium (Ca^{2+}) is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (1). Cells generate Ca^{2+} signals through both internal and external Ca^{2+} sources. In most cell types, the major internal Ca^{2+} stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the classical phosphoinositide pathway. Essentially, the binding of many hormones to specific receptors on the plasma membrane leads to the activation of an enzyme (phosphoinositidase C) that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate ($InsP_3$). Although derived from a lipid, $InsP_3$ is water soluble and diffuses into the cell interior where it encounters $InsP_3$ receptors ($InsP_3Rs$) on the ER/SR. The binding of $InsP_3$ changes the conformation of $InsP_3Rs$ such that an integral channel is opened, thus allowing the Ca^{2+} stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of $InsP_3Rs$ is that their opening is regulated by the cytosolic Ca^{2+} concentration. This sensitivity to cytosolic Ca^{2+} allows them to act as Ca^{2+}-induced Ca^{2+} release (CICR) channels that promote the rapid amplification of smaller trigger events (1).

It was thought that the binding of $InsP_3$ was obligatory for channel opening. However, in an elegant study in this issue of PNAS, Foskett and colleagues (2) demonstrate that a protein could supplant the need for $InsP_3$. Using a yeast two-hybrid screen, those authors demonstrate a high-affinity interaction between the NH_2-terminal 600 aa of an $InsP_3R$ and a member of a previously cloned group of proteins called Ca^{2+} binding proteins (CaBPs; ref. 3). These proteins belong to a superfamily of proteins that includes the well-known protein calmodulin, which bind Ca^{2+} by EF-hand motifs. CaBPs belong to a subfamily of EF-hand-containing proteins known as neuronal calcium sensors (NCS) (4). Although members of this group of proteins show great structural similarities, they have very diverse functional properties. The NCS subfamily consists of small proteins (molecular mass ≈20 kDa) that are exclusively expressed in neurons or retinal photoreceptors. They have a high degree of sequence similarity to calmodulin, but differ with respect to the number of functional Ca^{2+}-binding EF hands. The proposed physiological roles of NCS proteins include modulation of neurotransmitter release and regulation of gene transcription (4).

The study by Foskett and colleagues (2) proposes a new function for the CaBP members of the NCS family—regulation of $InsP_3Rs$ and Ca^{2+} release from intracellular stores. CaBPs interacted with $InsP_3Rs$ only in the amino-terminal region that was used for bait, which also contains the domain where $InsP_3$ itself binds to the channel. Patch-clamp recording of single $InsP_3Rs$ in the outer nuclear membrane of *Xenopus* oocytes indicated that CaBPs could activate the channels in the presence or absence of $InsP_3$. In fact, CaBP alone gave rise to substantial opening of the $InsP_3Rs$ (P_o ≈0.8 in optimal conditions), with significant gating observed at 10 nM CaBP. The interaction between CaBPs and $InsP_3Rs$ was greatly potentiated by increasing cytosolic Ca^{2+} over its physiological range (0.1–1 μM). This process critically depended on the EF hands, because mutations that inhibited Ca^{2+} binding to these domains abrogated the ability of CaBP to bind and activate $InsP_3Rs$.

CaBPs can act as endogenous high-affinity ligands of $InsP_3Rs$.

The picture that emerges is that CaBPs can act as endogenous high-affinity ligands of $InsP_3Rs$, which can activate Ca^{2+} release without the need for $InsP_3$ production via the phosphoinositide pathway (see Fig. 1). Because the action of CaBPs depends on Ca^{2+} binding, $InsP_3R$ signaling would still operate in a CICR mode; CaBPs can act on $InsP_3Rs$ to potentiate trigger Ca^{2+} signals arising from other sources, but presumably cannot initiate Ca^{2+} transients. At least five different CaBP isoforms with multiple splice variants exist, and they appear to have a clear tissue-specific expression profile (3). Some of the isoforms are abundantly expressed in the cerebral cortex and hippocampus, suggesting that CaBPs may be key mediators of Ca^{2+} signaling in the brain.

Although we can presently only speculate on the functions of CaBPs in neuronal Ca^{2+} signaling, their subcellular localization suggests some intriguing possibilities. Cellular fractionation and immunohistochemistry of CaBP-expressing neurons suggests that these proteins are present in synaptic and dendritic compartments, where $InsP_3Rs$ are also located. These are strategic locations where information processing occurs. If CaBPs can participate in Ca^{2+} signals occurring during synaptic signaling they may have an important role in memory formation. Ca^{2+} signaling within spines underlie changes in synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are models for learning and memory. Ca^{2+} signals underlie synaptic plasticity, and it is well established that $InsP_3Rs$ are involved in some of these paradigms (5, 6). It has been proposed that $InsP_3Rs$ act as "coincidence detectors" to interpret simultaneous signals arising from metabotropic receptors (producing $InsP_3$) and the Ca^{2+} entry through voltage-operated Ca^{2+} channel and receptor-operated Ca^{2+} channels

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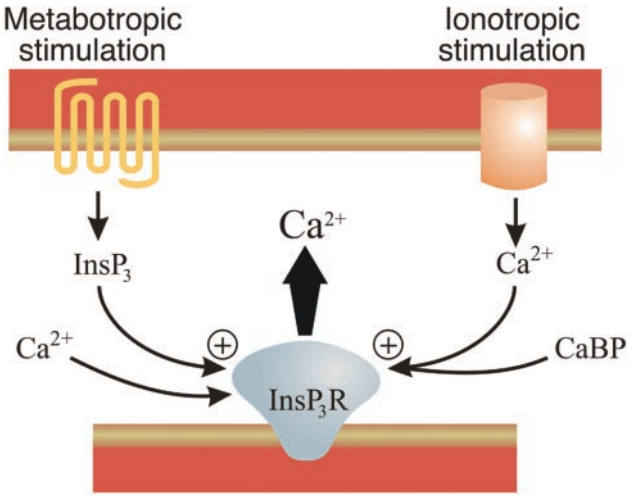

Fig. 1. Dual pathways for activation of $InsP_3Rs$. Shown are the alternative pathways leading to $InsP_3R$ activation in neurons expressing CaBPs. Metabotropic receptors lead to $InsP_3$ production via phospholipase C, whereas ionotropic stimulation produces an influx of Ca^{2+} that engages CaBP-bound $InsP_3Rs$ or recruits CaBP to the $InsP_3R$. Both metabotropic and ionotropic pathways lead to the activation of $InsP_3Rs$. $InsP_3$ requires the concurrent binding of Ca^{2+} to an activatory site on the $InsP_3R$. It is presently unclear whether the Ca^{2+}-bound form CaBP is sufficient for $InsP_3R$ opening, or if Ca^{2+} is also required to bind to $InsP_3Rs$ independently.

(such as *N*-methyl-D-aspartate receptors) (7). CaBPs could obviate the need for metabotropic stimulation during LTD/LTP, thus enabling $InsP_3Rs$ to participate in information processing in the absence of metabotropic receptor activation. The versatility of $InsP_3Rs$ would be enhanced through this responsiveness to $InsP_3$ or CaBP, thus enabling them to operate after activation of metabotropic or ionotropic mechanisms.

Unless signals become amplified by CICR, buffering and sequestration usually determines that Ca^{2+} has a very limited diffusion within the cytosol of most cells. $InsP_3$ is more freely diffusible, although it is typically metabolized to the non-Ca^{2+} releasing messengers inositol 1,4-bisphosphate ($InsP_2$) and inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) within seconds. The enzyme that catalyses the latter reaction to produce $InsP_4$, $InsP_3$3-kinase, has been proposed to act as a "firewall" within spines to stop the spread of $InsP_3$ into dendritic shafts and neighboring spines (8). If Ca^{2+}-bound CaBP can act as a mobile signal, it may smuggle an activatory signal beyond the barrier that prevents $InsP_3$ diffusion, to allow $InsP_3R$-dependent Ca^{2+} signaling over significant distances. An analogous situation occurs with calmodulin, which can diffuse over tens of micrometers within cells to allow local Ca^{2+} signals to activate gene transcription (9, 10).

Just how mobile CaBPs are is not fully resolved. One feature of the NCS family is that its members contain consensus sequences for myristoylation (4). This posttranslational modification results in Ca^{2+}-dependent translocation of these proteins, although along distinct routes (11). Expression of isoform variants of a CaBP in Chinese hamster ovary cells revealed that it could have distinct cellular localizations—a short form was found at or near the plasma membrane, whereas a longer homologue was associated with the cytoskeleton (3). The ability of CaBPs to bind and activate $InsP_3Rs$ would clearly be altered if they were tethered by lipid groups or structural proteins distant from $InsP_3Rs$.

Although the study from Foskett and colleagues (2) clearly demonstrates that CaBPs can regulate the activation of $InsP_3Rs$, there are clearly many outstanding questions concerning their mode of action. $InsP_3$ can have complicated effects on $InsP_3Rs$; it has been demonstrated to cause intrinsic inactivation of $InsP_3Rs$ and at high concentrations to protect them from Ca^{2+}-dependent inhibition (12). It will be interesting to see whether CaBPs can replicate all of the effects of $InsP_3$ on channel gating. Furthermore, it is possible that activation of $InsP_3Rs$ is not the sole function of CaBPs. In common with other members of the NCS subfamily, they appear to interact with several targets (4). CaBPs have been demonstrated to activate calmodulin-dependent kinase II and G protein-coupled receptor kinases *in vitro* (3). Whether such interactions occur *in vivo* is not yet known, however, it points to a potential pleiotropic action of the CaBPs after a Ca^{2+} signal. In addition, the CaBPs have consensus sequences for phosphorylation and have been shown to be phosphorylated in hippocampal slices *in situ* (13). Their functions could therefore be modulated by upstream kinases. Another possibility is that the expression of CaBPs could be modulated in a developmental- or activity-dependent manner, in which case their effects on the $InsP_3Rs$ could vary substantially.

For the activation of calmodulin-dependent kinase II and G protein-coupled receptor kinases, CaBPs essentially mimicked the role of calmodulin (3). Interestingly, $InsP_3Rs$ also have calmodulin binding regions, which can modulate the activity of the channels (14–16). One of the calmodulin binding sites has been mapped to the NH2-terminal 159 aa of the $InsP_3R$ (17), which is within the "bait" region used by Yang *et al.* (2) in their yeast two-hybrid screen. However, in their study, calmodulin had little effect on the interaction of CaBPs with $InsP_3Rs$, suggesting that they have distinct binding sites. In addition, calmodulin did not activate $InsP_3R$ channel activity in membrane patches. Therefore, the effects of CaBP and calmodulin on $InsP_3Rs$ appear to occur through distinct binding sites. It is plausible that these two proteins may have opposite effects on the opening of $InsP_3Rs$, with CaBPs causing Ca^{2+}-dependent activation and calmodulin evoking Ca^{2+}-dependent inhibition.

CaBPs are not the only members of the NCS family that regulate intracellular Ca^{2+} signals. Neuronal Ca^{2+} sensor 1/frequenin has been shown to activate phosphatidylinositol 4-kinase (18) and facilitate the activation of P/Q-type voltage-operated Ca^{2+} channels (19). Therefore, several of the NCS proteins have the capacity to both interpret and modulate neuronal Ca^{2+} signals.

The demonstration that CaBPs can activate $InsP_3Rs$ in the absence of $InsP_3$ raises many important questions for future work. CaBPs essentially endow $InsP_3Rs$ with the ability to display CICR without $InsP_3$ binding. This would make them functionally equivalent to ryandodine receptors (RyRs), which are intracellular Ca^{2+} release channels directly gated by Ca^{2+}. Neurons expressing CaBPs, $InsP_3Rs$, and RyRs would therefore possess two channels capable of autonomous CICR and potentially causing steeply regenerative Ca^{2+} signals. It will be important to find out what decelerates CaBP-induced Ca^{2+} release to prevent it from simply running away with itself. Additional questions include the localization and translocation of CaBPs. Does this limit or enhance their effects on $InsP_3Rs$? The observation that CaBPs can bind to $InsP_3Rs$ at low Ca^{2+} concentration (2) suggests that the Ca^{2+}-free forms of these proteins can be prebound to $InsP_3Rs$. This may be a way of speeding up the responsiveness of $InsP_3Rs$, in a similar way that complexing apo-calmodulin does for P/Q-type voltage-

operated Ca^{2+} channels (20). Finally, are $InsP_3Rs$ engaged with CaBP still responsive to $InsP_3$, or do CaBPs completely decouple the phosphoinositide signaling pathway from Ca^{2+} release? Clearly, the novel function for CaBPs proposed by Foskett and colleagues (2) opens up a new concept in the regulation of Ca^{2+} release.

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